Crystallographic Structure of a HslUV Complex

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INTRODUCTION

The HslUV complex (for heat shock locus gene products U and V [1]) is a bacterial homolog of the eukaryotic proteasome [2-4] which degrades specific target proteins in an ATP-dependent, chaperone-assisted manner. The protease component, HslV, is a "double donut" of hexameric rings, with the peptidase catalytic sites located in an interior cavity of the complex [5, 6]. Structures of the *E. coli* HslV protease [7] and the structurally related archaeal *Thermoplasma acidophilum* [8] and eukaryotic yeast [9] proteasomes are known.

By itself, HslV has a relatively low peptidase activity on short peptide substrates and negligible "polypeptidase" activity on protein substrates; the proteolytic activity of HslV is enhanced one to two orders of magnitude by its cognate chaperone HslU, hexameric rings of which bind HslV to form the active HslUV complex [10-12]. HslU is a member of the "Clp/Hsp100" family of molecular chaperones [13], as well as a member of the extended AAA (for ATPases associated with a variety of cellular activities [14]) family, a group of proteins whose diverse activities often require ATP-modulated assembly of oligomeric ring structures.

During the past year, we solved the structure of the *Haemophilus influenzae* HslUV complex to a resolution of 3.4 Ångstrom. In contrast to a structure which has been reported for the *E. coli* HslUV complex [15], our structure reveals a mode of quaternary assembly which is consistent with data from electron microscopy and solution small-angle x-ray scattering, and which suggests a mechanism of allosteric activation of the protease by the chaperone.

EXPERIMENTAL METHODS

Crystallographic data were collected on synchrotron beamline 5.0.2 of the Lawrence Berkeley Laboratory Advanced Light Source (ALS). Crystals were first adapted stepwise over a period of hours to a stabilization solution: 20% PEG-MME_{2K}, 1 M KCl, 50 mM citrate, 10 mM $Mg(OAc)_2$, 1 mM ATP, pH 6.0. Then, the 1 M KCl of the stabilization solution was replaced stepwise with 1 M $Mg(OAc)_2$, which acted as a cryoprotectant, allowing the crystals to be flash-frozen in a stream of nitrogen gas at ~100 K. Crystals are orthorhombic, space group $P2_12_12$, a = 209.22 Å, b = 220.58 Å, c = 241.07 Å after freezing, with one ~820 kDa $U_{12}V_{12}$ complex per asymmetric unit. Diffraction is anisotropic, with the weakest diffraction along the c* reciprocal space axis. Data were collected on an ADSC CCD detector and processed with DENZO; for native HslUV crystals, λ= 1.10000 Å ; for HslUV crystals incorporating SeMet-HslU, λ = 0.97992 Å, the absorption peak of Se in the crystals. The structure of the HslUV complex was solved by molecular replacement with models of the uncomplexed hexameric HslU and dodecameric HslV oligomers. The final molecular model was corroborated with sites of the selenium atoms determined from an anomalous difference Fourier on data from crystals of HslUV with SeMet-HslU. Data collection and refinement statistics are summarized in Table 1.

RESULTS

The structure of the HslUV complex is shown in the schematic drawing. Two hexameric ATP-binding rings of HslU (top and bottom in figure) bind intimately to opposite sides of the HslV protease; the HslU "intermediate domains", most of which are disordered in the crystal and hence not included in the model, extend outward from the complex. When the complex forms, the carboxy terminal helices of HslU distend and bind between subunits of HslV. Also, the apical helices of HslV shift substantially, transmitting a conformational change to the active site region of the protease, presumably resulting in the allosteric activation of its peptidase activity. The structure suggests a model whereby the ATP-binding, hexameric ring of HslU may be responsible for the allosteric activation of the protease, and the intermediate domains may be responsible for binding and initial unfolding of polypeptide substrates.

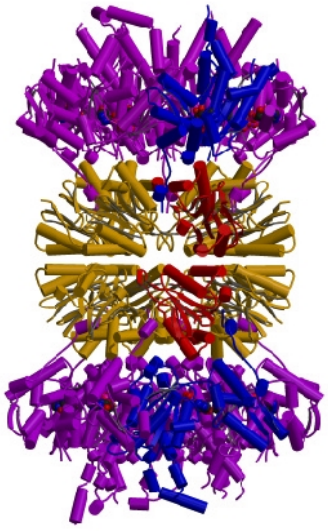

Table 1. Data collection and refinement statistics.

DATA COLLECTION		
Observations	297,864	
Unique reflections	134,912	
Resolution range (Å) (last shell)	30.0-3.40	(3.52-3.40)
Completeness	0.891	(0.806)
Av. Redundancy	2.2	(1.8)
Average I/σ	11.5	(3.0)
R_{sym} [a]	0.064	(0.285)
REFINEMENT		
Final resolution in refinement	30.0<->3.4	
R_{cryst} [b] (30-3.4)	0.241	
R_{free} [b] (30-3.4)	0.285	
Number of reflections (working set)	120,431	
Number of reflections (test set)	6,383	
Protein+ATP atoms	45,556	
rms bond deviation (Å)	0.010	
rms angle deviation (deg)	1.42	

Numbers in parentheses correspond to the last resolution shell. [a] $R_{sym} = \sum|I_i - \langle I \rangle| / \sum \langle I \rangle$ where I=diffraction intensity; <I>=mean measured intensity. [b] $R_{cryst} = \sum|F_c - F_o| / \sum F_o$ where F_o=observed structure factor amplitude in working set = $|I^{1/2}|$; F_c=structure factor amplitude calculated from model; R_{free} same, using F_o in test set (5.0% of reflections).

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